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Abstract: **BACKGROUND:** Activated phosphoinositide 3-kinase syndrome (APDS) 2 (p110-activating mutations causing senescent T cells, lymphadenopathy, and immunodeficiency [PASLI]-R1), a recently described primary immunodeficiency, results from autosomal dominant mutations in PIK3R1, the gene encoding the regulatory subunit (p85, p55, and p50) of class IA phosphoinositide 3-kinases. **OBJECTIVES:** We sought to review the clinical, immunologic, and histopathologic phenotypes of APDS2 in a genetically defined international patient cohort. **METHODS:** The medical and biological records of 36 patients with genetically diagnosed APDS2 were collected and reviewed. **RESULTS:** Mutations within splice acceptor and donor sites of exon 11 of the PIK3R1 gene lead to APDS2. Recurrent upper respiratory tract infections (100%), pneumonitis (71%), and chronic lymphoproliferation (89%, including adenopathy [75%], splenomegaly [43%], and upper respiratory tract lymphoid hyperplasia [48%]) were the most common features. Growth retardation was frequently noticed (45%). Other complications were mild neurodevelopmental delay (31%); malignant diseases (28%), most of them being B-cell lymphomas; autoimmunity (17%); bronchiectasis (18%); and chronic diarrhea (24%). Decreased serum IgA and IgG levels (87%), increased IgM levels (58%), B-cell lymphopenia (88%) associated with an increased frequency of transitional B cells (93%), and decreased numbers of naive CD4 and naive CD8 cells but increased numbers of CD8 effector/memory T cells were predominant immunologic features. The majority of patients (89%) received immunoglobulin replacement; 3 patients were treated with rituximab, and 6 were treated with rapamycin initiated after diagnosis of APDS2. Five patients died from APDS2-related complications. **CONCLUSION:** APDS2 is a combined immunodeficiency with a variable clinical phenotype. Complications are frequent, such as severe bacterial and viral infections, lymphoproliferation, and lymphoma similar to APDS1/PASLI-CD. Immunoglobulin replacement therapy, rapamycin, and, likely in the near future, selective phosphoinositide 3-kinase inhibitors are possible treatment options.

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Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase δ syndrome 2: A cohort study



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Background: Activated phosphoinositide 3-kinase δ syndrome (APDS) 2 (p110 δ -activating mutations causing senescent T cells, lymphadenopathy, and immunodeficiency [PASLI–R1], a recently described primary immunodeficiency, results from autosomal dominant mutations in *PIK3R1*, the gene encoding the regulatory subunit (p85 α , p55 α , and p50 α) of class IA phosphoinositide 3-kinases.

Objectives: We sought to review the clinical, immunologic, and histopathologic phenotypes of APDS2 in a genetically defined international patient cohort.

Methods: The medical and biological records of 36 patients with genetically diagnosed APDS2 were collected and reviewed.

Results: Mutations within splice acceptor and donor sites of exon 11 of the *PIK3R1* gene lead to APDS2. Recurrent upper respiratory tract infections (100%), pneumonitis (71%), and chronic lymphoproliferation (89%, including adenopathy [75%], splenomegaly [43%], and upper respiratory tract lymphoid hyperplasia [48%]) were the most common features.

Growth retardation was frequently noticed (45%). Other complications were mild neurodevelopmental delay (31%); malignant diseases (28%), most of them being B-cell lymphomas; autoimmunity (17%); bronchiectasis (18%); and chronic diarrhea (24%). Decreased serum IgA and IgG levels (87%), increased IgM levels (58%), B-cell lymphopenia (88%) associated with an increased frequency of transitional B cells (93%), and decreased numbers of naive CD4 and naive CD8 cells but increased numbers of CD8 effector/memory T cells were predominant immunologic features. The majority of patients (89%) received immunoglobulin replacement; 3 patients were treated with rituximab, and 6 were treated with rapamycin initiated after diagnosis of APDS2. Five patients died from APDS2-related complications.

Conclusion: APDS2 is a combined immunodeficiency with a variable clinical phenotype. Complications are frequent, such as severe bacterial and viral infections, lymphoproliferation, and lymphoma similar to APDS1/PASLI–

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Key words: Primary immunodeficiency, phosphoinositide 3-kinase, p85 α , p110 δ , activated phosphoinositide 3-kinase δ syndrome, p110 δ -activating mutations causing senescent T cells, lymphadenopathy, and immunodeficiency, hyper-IgM, adenopathy, immunodeficiency, antibody deficiency

Activated phosphoinositide 3-kinase δ syndrome (APDS) 2, also called p110 δ -activating mutations causing senescent T cells, lymphadenopathy, and immunodeficiency (PASLI-R1 [MIM# 616005]), is a primary immunodeficiency resulting from autosomal dominant mutations in *PIK3R1*, the gene encoding the regulatory subunit (p85 α , p55 α , and p50 α) of class IA phosphoinositide 3 kinases (PI3Ks).^{1,2} Class IA PI3K molecules are composed of a p110 catalytic subunit (p110 α , p110 β , or p110 δ) and a regulatory subunit (p85 α , p55 α , p50 α , p85 β , or p55 γ) that regulates the stability, cellular localization, and function of p110. The function of class IA PI3Ks is to convert phosphatidylinositol 4,5-bisphosphate into phosphatidylinositol 3,4,5-trisphosphate, an important phospholipid secondary messenger. Each of the catalytic subunits can bind to any of the regulatory subunits.³ Expression of the p110 δ catalytic subunit is restricted mainly to leukocytes, whereas p110 α and p110 β are ubiquitously expressed. The widely expressed p85 α regulatory subunit is the predominant regulatory subunit in lymphocytes. Mutations in a splice donor site of *PIK3R1* have been shown to cause APDS2 as a result of skipping of exon 11 (coding exon 10), encoding amino acids 434 to 475 of p85 α . Splicing from exon 10 to exon 12 is in frame and therefore results in a shortened p85 α protein; the p55 α and p50 α isoforms are similarly affected.

Abbreviations used

APDS: Activated phosphoinositide 3-kinase δ syndrome
CHL: Classical Hodgkin lymphoma
CMV: Cytomegalovirus
DLBCL: Diffuse large B-cell lymphoma
ENT: Ear, nose, and throat
HIGM: Hyper-IgM
HSCT: Hematopoietic stem cell transplantation
PASLI: p110 δ -Activating mutations causing senescent T cells, lymphadenopathy, and immunodeficiency
PI3K: Phosphoinositide 3-kinase

The shortened p85 α protein is dominantly responsible for hyperactivated PI3K δ signaling in T and B lymphocytes.^{1,2}

The main clinical and biological findings in the 13 published patients with APDS2 reported thus far were recurrent respiratory tract infections, lymphoproliferation, and antibody deficiency.^{1,2,4,5} APDS2 resembles APDS1, which is also named PASLI-CD (MIM# 615513), a primary immunodeficiency caused by autosomal dominant gain-of-function mutations in *PIK3CD*, the gene encoding the catalytic subunit p110 δ , leading to hyperactivated PI3K δ signaling in lymphocytes.⁶⁻⁸

In this study we reviewed the clinical, immunologic, and histopathologic features of APDS2 in a genetically defined international cohort of 36 patients.

METHODS

Genomic DNA from patients presenting with genetically undefined primary antibody deficiency was screened for mutations at the splice sites of exon 11 (coding exon 10) of the *PIK3R1* gene by using whole-exome sequencing or targeted Sanger sequencing. Medical and biological records

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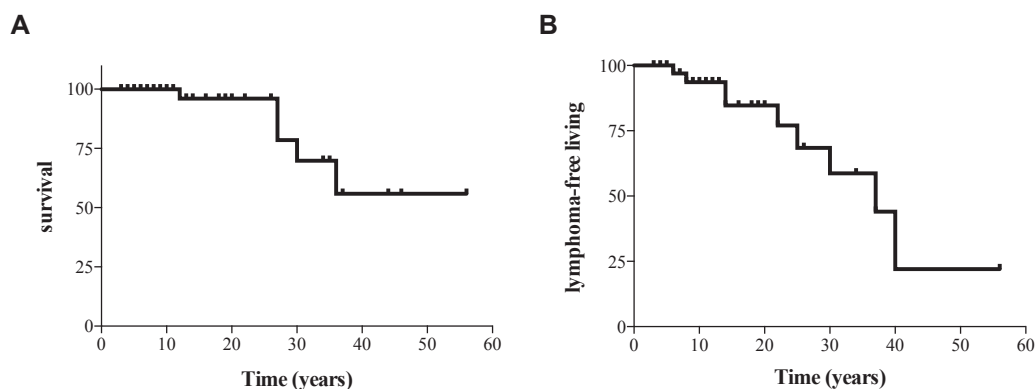


FIG 1. Overall survival and lymphoma-free living of patients with APDS2. **A**, Overall survival of patients with APDS2 from the cohort. **B**, Cumulative risk of lymphoma according to age. The time of lymphoma-free life in patients with APDS2 is depicted.

of 36 patients with genetically diagnosed APDS2 were retrospectively collected and compared by using a questionnaire. Patients treated for lymphoma, receiving rituximab, or both were excluded from the immunologic analysis. The study was performed in accordance with the precepts of the Declaration of Helsinki and local ethical requirements.

RESULTS

Patients' characteristics

In this retrospective analysis 36 patients with APDS2 (15 male patients) from 31 unrelated families were included, 8 of whom were reported previously.^{1,2} Five patients died at the age of 12 (P10), 27 (P5 and P28), 30 (P11), and 36 (P20a) years, respectively (Fig 1). Alive patients had a median age of 18 years (range, 3–56 years) at the time of the medical report.

Genetics of heterozygous splice site mutations in *PIK3R1*

The previously described G to A, G to C, and G to T nucleotide substitutions at the +1 position of the donor splice site of *PIK3R1* were identified in 42%, 29%, and 13% of the patients, respectively (Fig 2). In 4 (13%) patients novel mutations affecting the +2 position of this donor splice site were identified, a T to A mutation (2 patients), a T to G substitution, and a TG deletion. In addition, a novel mutation, a G to C nucleotide substitution at the –1 position of the splice acceptor site of exon 11 of the *PIK3R1* gene was identified (Fig 2). Exon skipping of exon 11 encoding amino acids 434 to 475 of p85 α was demonstrated by using mRNA analysis for all novel mutations (see Fig E1 in this article's Online Repository at www.jacionline.org).

Ten patients were familial cases (5 families), but the large majority of patients were sporadic cases. Analysis of DNA from parents was only available for 8 patients from sporadic cases and revealed *de novo* mutations.

Clinical presentation

Infectious complications. Clinical manifestations of the 36 patients are shown in Fig 3. All presented with early-onset recurrent ear, nose, and throat (ENT) or bronchopulmonary infections (median onset, 1.7 years of age; range, first month of life to 10 years of age). Upper respiratory tract (otitis media and sinusitis) and lower respiratory tract (bronchitis and pneumonitis) infections were present in 100% and 77% of

patients, respectively. Mild bronchial wall thickening on chest computed tomographic scans and bronchiectasis were noticed in 2 (6%) and 6 patients (18%), respectively, and bronchiectasis was diagnosed at a median age of 13 years (range, 4–33 years). The most common bacterial respiratory organisms identified were *Haemophilus influenzae* and *Streptococcus pneumoniae*. Chronic conjunctivitis reported in 7 patients progressed in 1 patient to *Staphylococcus aureus*-related periorbital cellulitis (P5b) and in 2 patients to chronic blepharitis (P16 and P27a). Invasive bacterial infections were rare, being reported only in 2 cases, 1 patient who presented with *Pseudomonas aeruginosa* septicemia (P20a) and a 12-year-old boy (P10) who had peritonitis related to infectious perforation of the small intestine, leading to septic shock and death. This boy had chronic gastroenteritis associated with *Campylobacter jejuni*, *Salmonella typhimurium*, and *Clostridium difficile* infections. Chronic cutaneomucosal candidiasis was observed in 3 patients (P5, P25, and P28). Of 17 patients who received BCG vaccination, 2 (P21 and P26) presented with persistent local skin lesions at the vaccination site. Persistent detection of virus was reported in 36% of patients, with cytomegalovirus (CMV) and EBV the most common. Disseminated lymphadenitis associated with CMV infection was reported in 2 patients, and asymptomatic chronic CMV viremia was detected in 6 (17%) patients. Chronic EBV viremia was detected in 8 (22%) patients and reported in 4 patients in combination with EBV-associated lymphoproliferative disease and in 4 patients as asymptomatic chronic EBV viremia. Severe varicella zoster virus infections requiring hospitalization occurred in 2 patients (P21 and P26). One patient had hydrocephalus after measles meningitis (P22). Two patients presented with localized molluscum contagiosum (P17 and P27a) and 1 patient presented with warts (P22), indicating pox virus and papilloma virus infections, respectively. Chronic viral hepatitis was reported in 3 patients, as related to either hepatitis B (P11, P20a) or C (P5) infection. Except chronic *Giardia intestinalis* in 1 patient (P5) and ocular toxoplasmosis in another (P20a), no other parasitic infections were reported in our patient cohort.

Lymphoproliferation. Thirty-two (89%) of 36 patients had persistent (>6 months) benign lymphoproliferation either as chronic lymphadenopathy, splenomegaly, or ENT or gut infiltration (Fig 3). Lymphadenopathy and splenomegaly typically began in childhood. Lymphadenopathies mentioned in

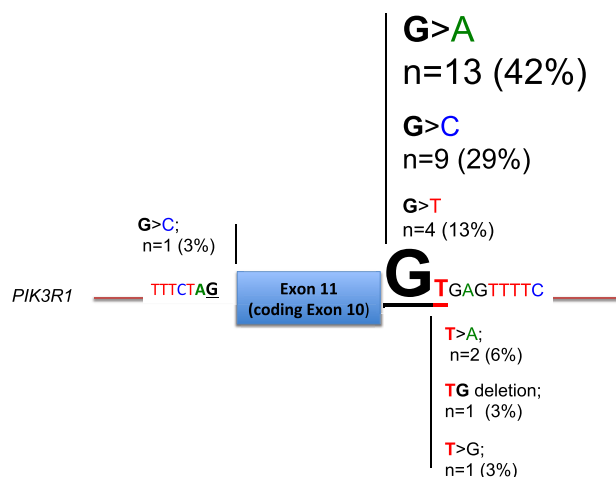


FIG 2. Confirmed heterozygous mutations in the *PIK3R1* gene of patients with APDS2. Frequency and number of patients carrying indicated mutations are presented. Mutations present in several patients from 1 family were counted as 1 mutation.

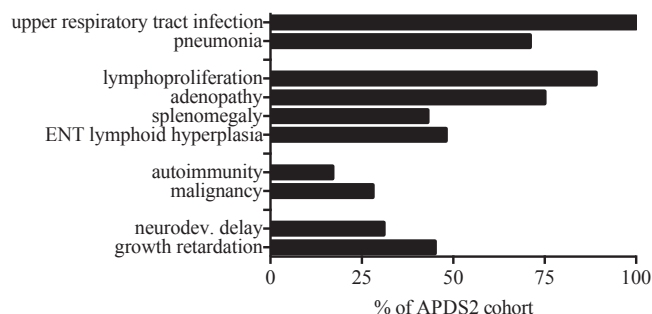


FIG 3. Main clinical characteristics of the APDS2 cohort. Shown is the percentage of patients who presented with the indicated clinical features. *neurodev. delay*, Neurodevelopmental delay.

75% of the patients were variable in size, from mild (1-3 cm) in 18 (50%) of 36 patients to large in 9 (25%) patients (3-5 cm, $n = 7$; >5 cm, $n = 2$) (Fig 4). Fifteen (43%) patients had splenomegaly of variable size (Figs 3 and 4). Hepatomegaly developed in 8 (22%) patients. Nodular lymphoid infiltration of the gut was reported in 8 (24%) patients and was associated with chronic diarrhea, malabsorption, or both. Severity of ENT infiltration was variable, ranging from ENT chronic lymphoid hyperplasia without the need for surgical interventions in 3 (11%) patients to adenoidectomies, tonsillectomy, or both in 7 (26%) patients to multiple surgical resections in 3 patients. One 6-year-old patient required multiple surgical interventions, including maxillary antrastomies, multiple adenoidectomies, tonsillectomies, and reductions of basalingual tonsils. The patient subsequently had postoperative pharyngeal stenosis, requiring 3 endoscopic dilations, which were inefficient, leading to tracheotomy.

Tonsil biopsy specimens from P1 and P2 were available. As shown in Fig 5 and Fig E2 in this article's Online Repository at www.jacionline.org (both presented identical abnormalities compared with age-matched control subjects): prominent T-cell hyperplasia and small B-cell follicles were noticed. Germinal centers were small and ill-defined, with very few IgD⁺ mantle cells. Large B cells in the interfollicular area were numerous, and IgM⁺ cells, which are usually localized in germinal centers,

were scattered within the T-cell zone. In addition, an important hyperplasia of PD1⁺ T cells was present both in germinal center and in extrafollicular areas. The frequency of scattered EBV⁺ cells, CMV⁺ cells, or both present in the patients' biopsy specimens were comparable with those observed in control biopsy specimens and not consistent with EBV-driven pathologies, CMV-driven pathologies, or both.

Lymphoma. Ten (28%) patients had malignant diseases (Fig 1, B, and Table I) at a median age of onset of 23 years (range, 6-40 years). The cumulative risk of lymphoid malignancy at the age of 40 years was calculated as 78% (Fig 1, B). Classical Hodgkin lymphoma (CHL) was diagnosed in 5 (14%) patients. Diffuse large B-cell lymphoma (DLBCL) was diagnosed in 4 (11%) patients, and marginal zone B-cell lymphoma was diagnosed in 2 (6%) patients. Three patients had multiple lymphomas. One patient (P12) first had a nodular sclerosis CHL at the age of 14 years that was treated with chemotherapy and at the age of 27 years had a DLBCL that was treated with intensive chemotherapy and autologous hematopoietic stem cell transplantation (HSCT). Another patient (P20a) had 2 cases of EBV⁺ nodular sclerosis CHL at 14 and 35 years of age and a marginal zone B-cell lymphoma at 19 years of age. Her brother (P20b) presented also with CHL when he was 8 years old. Overall, 4 patients died of lymphoma at the ages of 27 (P5 and P28; DLBCL), 30 (P11; CHL), and 36 (P20a; CHL) years, respectively. Chronic lymphocytic leukemia developed in 1 patient (P27a) at 40 years of age. No other malignancy has been reported, except a papillary neoplasm in both breasts in a female patient.

Autoimmunity and immune dysregulation. Six (17%) patients had autoimmune complications. Two patients had thrombocytopenic purpura during childhood. One patient had autoimmune hemolytic anemia after chemotherapy for lymphoma, and 1 patient had Evans syndrome associated with chronic lymphocytic leukemia. Insulin-dependent diabetes was diagnosed in 1 patient. Two patients had chronic arthritis, and 1 had autoimmune hepatitis. In addition, 3 patients presented with chronic eczema.

Immunologic features

The patients' main immunologic characteristics are summarized in Fig 6, A-H (immunologic data for individual patients are provided in Tables E1 to E4 in this article's Online Repository at www.jacionline.org). The majority of patients presented with decreased serum IgG and IgA levels before onset of immunoglobulin replacement therapy (87%). Increased IgM levels were observed in most (58%) but not all patients because 26% presented with normal levels and 16% presented with decreased levels before any treatment. IgM levels decreased in 5 patients and increased in 2 patients over 2 to 12 years after onset of immunoglobulin replacement therapy. One patient (P3a) had increased IgG and IgM but decreased IgA levels; 1 (P28) had low IgA but normal IgG and IgM levels; and 1 (P4a) had increased IgA, decreased IgG, and normal IgM levels.

The majority of patients (88%) presented with B-cell lymphopenia worsening within 1 to 19 years (Fig 6, D, and see Fig E3 in this article's Online Repository at www.jacionline.org). Transitional B cells were increased in frequency in 14 (93%) of 15 patients who had a suitable number of CD19⁺ cells for analysis. Total CD3 T-cell counts were normal in 74% of patients

A Clinical features of APDS2 patients

Patient	%	1	2	3a	3b	4a	4b	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20a	20b	21	22	23a	23b	24	25	26	27a	27b	28	29	30	31
Age at medical report (yrs)	18*	13	7	37	5	56	19	27	26	20	6	11	12	30	35	19	16	34	9	11	14	18	36	26	7	34	18	44	18	8	4	46	16	27	10	3	22
Upper respiratory infections	100																																				
Pneumonia	71																																				
Bronchiectasis	18																																				
Autoimmunity	17																																				
Chronic diarrhea	24																																				
Adenopathy	75																																				
Splenomegaly	43																																				
Malignant disease	28																																				
Neurodevelopmental delay	31																																				
Growth retardation	45																																				
Dead	14																																				

B Biological features

Patient	%	1	2	3a	3b	4a	4b	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20a	20b	21	22	23a	23b	24	25	26	27a	27b	28	29	30	31
Increased IgM	58																																				
Decreased IgA/IgG	87																																				
EBV chronic replication	22																																				
CMV chronic replication	17																																				
Inverted ratio CD4/CD8	82																																				

C variability of lymphoproliferation

Patient	1	2	3a	3b	4a	4b	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20a	20b	21	22	23a	23b	24	25	26	27a	27b	28	29	30	31
ENT lymphoid hyperplasia																																				
Splenomegaly																																				
Adenopathy																																				

FIG 4. Main clinical complications and biological features of patients with APDS2. **A**, Clinical features of patients with APDS2. **B**, Biological features. *Median age at medical report of alive patients: red, affected; light yellow, unaffected; boxes with a diagonal, unknown. **C**, Variability of lymphoproliferation: light yellow, unaffected; boxes with a diagonal, unknown; dark yellow, ENT chronic lymphoid hyperplasia without the need of surgical interventions, lymphadenopathies with lymph node sizes from 1 to 3 cm; orange, adenoidectomies and/or tonsillectomy, lymph node sizes from 3 to 5 cm; red, multiple surgical resections, lymph node sizes larger than 5 cm. Splenomegaly was graded by its size: light yellow, not present, dark yellow, up to half distance between the costal margin and umbilicus; dark orange, up to the umbilicus; red, above the umbilicus.

(see Fig E4 in this article's Online Repository at www.jacionline.org), CD4 T-cell counts were normal in 67% of patients, and CD8 T-cell counts were increased in 52% of patients and remained stable over time. An inverted CD4/CD8 ratio (<1.0) was found in 82% of patients. When extended naive/memory T-cell phenotype analysis was performed, the increased CD8 T-cell numbers appeared to result from the expanded CD8 T-cell population with an effector/memory phenotype. Nearly all patients analyzed presented with a low number of naive CD4 T cells (CD31⁺CD45RA⁺/CD4⁺ cells; 71% of patients) and naive CD8 T cells (CCR7⁺CD45RA⁺/CD8⁺; 100% of patients), worsening over time (Fig 6, F and H, and see Fig E5 in this article's Online Repository at www.jacionline.org).

Nonimmunologic features

Growth impairment (−2 SDs of height) was found in 14 (45%) of 31 patients, a feature not always related to chronic diarrhea because it was absent in 9 of them. Height and weight were similarly affected because body mass index was within the normal range in all but 2 patients (minimum, −2.8 SD; maximum, +3.3 SD; median, −0.7 SD). Microcephaly was reported in 2 patients. Neurodevelopmental delay presenting as mild cognitive impairment or learning disabilities was reported in 9 (31%) patients. For 1 patient, extensibility of the joints and increased glucose levels in the blood were also reported. Liver cysts and polycystic kidneys were reported in 1 patient each.

Treatment

Twenty-two patients received various antibiotic prophylaxis (trimethoprim/sulfamethoxazole or azithromycin). The majority of patients (89%) received immunoglobulin replacement therapy

(median age at onset of treatment, 5 years; range, 1-35 years). Five patients were treated with steroids because of autoimmune cytopenia (n = 2) or lymphoproliferation (n = 3). Three patients were treated with rituximab to treat lymphoproliferation (n = 2) or autoimmune hemolytic anemia (n = 1). Three patients were splenectomized, 2 for autoimmune cytopenia and 1 as a diagnostic procedure of massive splenomegaly. Immunosuppressive drugs for digestive tract disease were given in 3 patients in different combination (azathioprine, mycophenolate mofetil, methotrexate, and infliximab). Episodes of lymphomas were treated conventionally with chemotherapy associated in some cases with radiotherapy and in 3 patients with autologous HSCT. Allogeneic HSCT from an HLA-matched (10/10) unrelated donor was performed in 1 patient (P27b) because of molecular diagnosis, recurrent infections, and family history. The conditioning regimen consisted of 42 g/m² treosulfan, 150 mg/m² fludarabine, and alemtuzumab. Five months after HSCT, the patient was alive and well, with 100% donor chimerism and no sign of GVHD. Since the diagnosis of APDS2, 6 patients were started on rapamycin treatment. The time of follow-up after onset of rapamycin treatment was too short to evaluate treatment efficacy for 4 patients. Two patients with APDS2 were doing well on rapamycin treatment. For both patients, significant reduction of lymphoproliferation was reported.

DISCUSSION

Our retrospective analysis comparing clinical features of patients with APDS2/PASLI-R1 indicated a highly heterogeneous clinical phenotype with recurrent ENT and bronchopulmonary infections during early childhood as the most common clinical manifestation. Chronic benign lymphoproliferative complications with various degrees of severity manifesting as

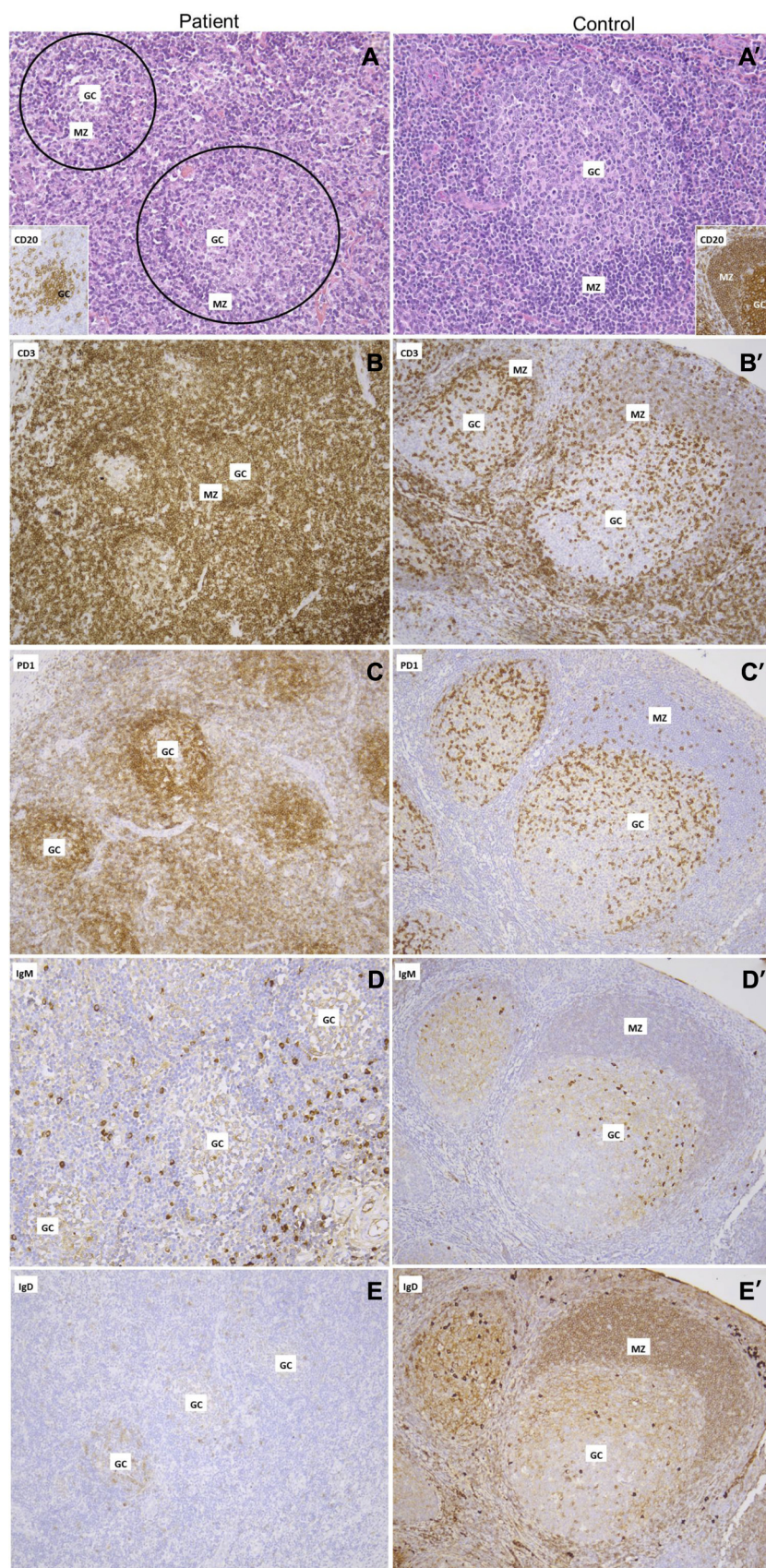


TABLE I. Malignant diseases

Patient ID	Age (y) at PID diagnosis	Age (y) at onset of cancer	Type of cancer	Dead/alive
P5	4	25	DLBCL	Dead
P11	22	30	CHL	Dead
P12	Infancy	14/27	CHL/DLBCL	Alive
P19	9	6/11	DLBCL/MALT	Alive
P20a	36	14/19/35	CHL/MZL/CHL	Dead
P20b	8	8	CHL	Alive
P22	6	30	Breast papillary neoplasm	Alive
P23b	Infancy	37	CHL	Alive
P27a	31	40	CLL	Alive
P28	5	22	DLBCL	Dead

CHL, Classical Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; MALT, mucosa-associated lymphoid tissue lymphoma; MZL, marginal zone B-cell lymphoma; PID, primary immunodeficiency.

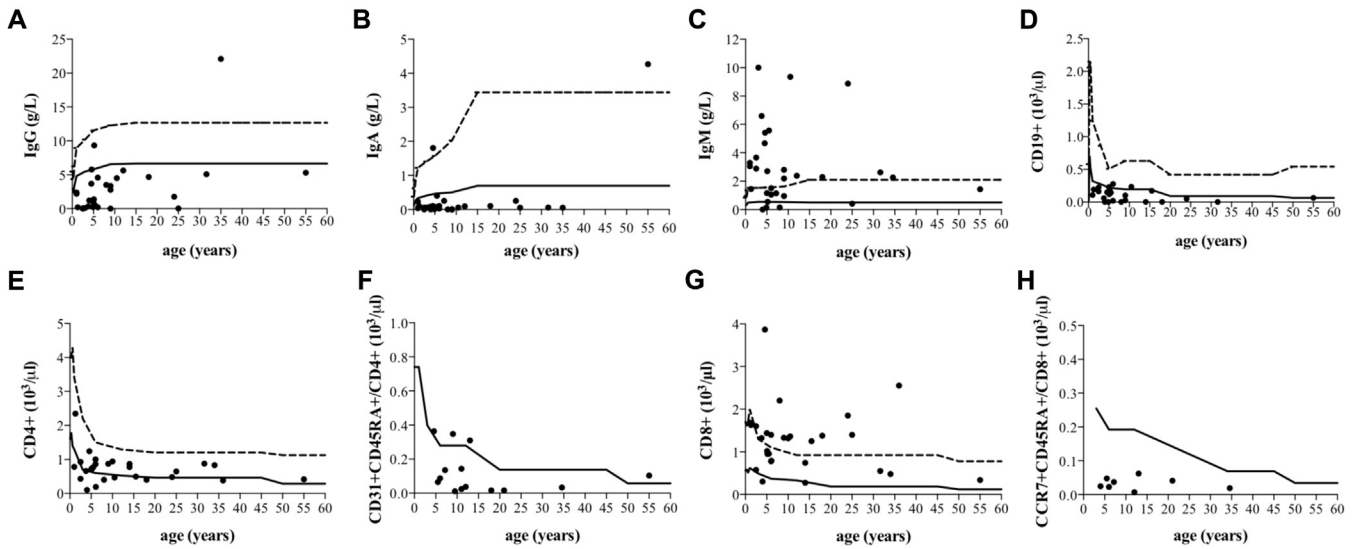


FIG 6. Immunologic features of patients with APDS2. **A-D**, IgG (Fig 6, A), IgA (Fig 6, B), and IgM levels (Fig 6, C) and B-cell numbers (Fig 6, D) of patients with APDS2 before onset of immunoglobulin replacement or other therapies. **E-H**, CD4 (Fig 6, E), CD4-naïve (Fig 6, F), CD8 (Fig 6, G), and CD8-naïve (Fig 6, H) T-cell subsets of patients with APDS2. Fig 6, E and G, indicate before onset of immunoglobulin replacement or other therapies and Fig 6, F and H, indicate the last evaluation. Solid line, Lower reference value; dashed line, upper reference value.

adenopathies, splenomegaly, or hepatomegaly were observed. Persistent EBV and/or CMV viremia was detected in several patients, indicating impaired control of viral infections. Predominant biological parameters included hyper-IgM (HIGM) features, B-cell lymphopenia associated with increased frequency of transitional B cells, decreased naïve CD4 and CD8 T-cell numbers, and increased cell number and frequency of CD8 effector/memory T cells. B-cell lymphoma, especially CHL, DLBCL, and marginal zone B-cell lymphoma, was frequently reported in our cohort, indicating the oncogenic character of these *PIK3R1* splice site mutations. Noninfectious and immunologic

manifestations noted in our APDS2 cohort were growth retardation and mild neurodevelopmental delay.

Overall, our work underscores the conclusion that APDS2 shares similarities with APDS1. Both syndromes include a predominant antibody deficiency frequently presenting as a hyper-IgM-like syndrome associated with progressive B- and naïve T-cell lymphopenia and massive lymphoproliferation. The phenotypic heterogeneity of patients with APDS2, similar to that observed in patients with APDS1, might be related to the patient’s history of infections, environmental factors, and/or the presence of modifier genes.

FIG 5. Histologic features of tonsil biopsy specimens from patients with APDS2. **A-E**, Patient with APDS2. **A'-E'**, Control subject. All pictures are at the same magnification (×10). GC, Germinal center; MZ, mantle zone. B-cell follicles are small (Fig 5, A: follicles defined by circles, B, C, D), with a few CD20⁺ B cells (inset CD20 staining Fig 5, A) compared with the control values (inset CD20 staining Fig 5, A') and associated with prominent CD3⁺ T-cell hyperplasia (Fig 5, B and B', anti-CD3 staining) compared with control values. PD1 staining underlines the important hyperplasia of germinal center and extrafollicular PD1⁺ T cells (Fig 5, C) compared with control cells (Fig 5, C'). Germinal centers are ill-defined (Fig 5, A-C), and IgM⁺ cells, which are usually localized in the germinal center (Fig 5, D', control), are scattered in the T-cell zone (Fig 5, D). Only a few residual IgD⁺ mantle cell zone cells are present (Fig 5, E) compared with control cells (Fig 5, E').

However, in contrast to APDS1 (Coulter et al, unpublished data), histologic analysis revealed a reduced germinal center size in the 2 available tonsil biopsy specimens. Although we cannot exclude that this observation could be due to the heterogeneous spectrum of the disease because only a limited number of biopsy specimens were available, it might suggest that deletion of exon 11 of the *PIK3R1* gene affects not only p110 δ but also other catalytic subunits of class IA PI3Ks.

Increased or normal IgM levels together with decreased IgG and IgA serum levels and B-cell lymphopenia associated with increased frequency of transitional B cells were frequently observed in patients with APDS2 and can be explained by an intrinsic B-cell defect leading to enhanced differentiation of B lymphocytes from patients with APDS2 into short-lived IgM-producing plasmablasts, as reported for phosphatase and tensin homolog (*Pten*)-deficient murine B cells.⁹ Our histologic analysis identifying numerous large IgM⁺ B cells located in the interfollicular area further supports this hypothesis. Moreover, our histologic analysis indicated that hyperactive PI3K signaling interferes with the germinal center structure, likely inhibiting immunoglobulin class-switch recombination. Impaired immunoglobulin class-switch recombination as a cause of disturbed germinal center architecture was indeed recently described in a murine model analyzing hyperactive PI3K signaling in germinal center B lymphocytes.¹⁰ The B-cell lymphopenia in the blood of patients with APDS2 could be explained by disturbed migration because B cells are proliferating in the lymph nodes, as indicated by our histologic analysis.

The major complication of patients with APDS2, as well as those with APDS1, is development of B-cell lymphoma (9/36 [25%]).^{8,11} Predisposition of APDS2 (as for APDS1) to B-cell lymphomagenesis could be due to several immunologic abnormalities, such as a defective T cell-mediated immune surveillance, uncontrolled B-cell activation and proliferation, or both. Because the histopathologic analysis indicated an important hyperplasia of PD1⁺ T cells, aberrant follicular helper T cell function could be considered an additional factor for promoting survival of neoplastic B cells, as previously suggested for follicular helper T cells present within the microenvironment of nodular lymphocyte predominant Hodgkin lymphoma and follicular lymphoma.¹² Oncogenic potential of *PIK3R1* mutations has been previously suggested by the presence of somatic mutations in *PIK3R1* in patients with Burkitt lymphomas¹³ and those with endometrioid and colon cancers,¹⁴ affecting amino acid residues 437 to 475 encoded by exon 11 (coding exon 10) of the *PIK3R1* gene. Moreover, in the Catalogue of Somatic Mutations in Cancer project, mutations affecting the +1 position (G to A and G to T) and +2 position (T to C and T to G) at the same splice acceptor site of the *PIK3R1* gene found mutated in patients with APDS2 have been recently annotated, a strong argument in favor of the oncogenic character of these APDS2 splice site mutations. These somatic mutations were found in patients with carcinoma located in the ovary, large intestine, and stomach and malignant melanoma underlining the possible oncogenic potential of those mutations not only for B-cell lymphoma but for other cell types, suggesting an impairment of the *PIK3R1* gene-encoded regulatory subunits not only on p110 δ (PI3K δ) activity. Growth impairment, joint extensibility, and increased glucose levels in the blood reported for 1 patient might reflect deregulated p110 α (and/or p110 β) activity. More research will be needed to characterize the possible effects of

the mutant p85 $\alpha^{\Delta 434-475}$ protein on different catalytic p110 subunits in other cells (nonlymphoid lineage cells), which could be hidden by the predominant immunologic phenotype.

Of note, heterozygous nonsynonymous germline mutations located especially within the C-terminal part of p85 α (downstream of amino acid 475) result in a rare autosomal dominant multisystem disease called SHORT syndrome described to be due to loss of PI3K activity.¹⁵⁻¹⁷ Patients with SHORT syndrome present with short stature; hyperextensibility of joints, hernia (inguinal), or both; ocular depression; Rieger anomaly; and teething delay.

Allogeneic HSCT for APDS2 was recently reported for 1 case.⁵ Herein we describe a second successful case similar to the 8 of 11 successful cases of allogeneic HSCT for APDS1 (Coulter et al, unpublished data). Thus allogeneic HSCT appears to be a treatment option for those with severe APDS2, especially in light of the increased risk of lymphoma development (Fig 1, B, and Table I), although no prognostic marker for lymphoma development has been identified thus far.

Most patients have received immunoglobulin replacement therapy since infancy to reduce the infection incidence. Since the diagnosis of APDS2, 6 patients were started on long-term rapamycin treatment based on knowledge that the serine/threonine kinase mammalian target of rapamycin is activated by PI3K signaling, and rapamycin treatment was reported to be beneficial in patients with APDS1 (personal observation).⁷ For 2 patients with APDS2 in our cohort, rapamycin treatment was beneficial. For 1 patient, treatment led to disappearance of chronic conjunctivitis and normalization of tonsil size, and for the other patient, treatment led to reduced lymph node, liver, and spleen size; however, the effect of this treatment on lymphocyte cell numbers and antibody titers over a longer time period has to be investigated further. Evaluation of the efficacy of rapamycin treatment on the other patients with APDS2 in our cohort was not possible because of the short treatment period. Although continuous rapamycin treatment might turn out to be very beneficial for patients with APDS2, it bears the risk of unwanted side effects outside the immune system.¹⁸ Because the hyperactivated PI3K signaling in APDS2 lymphocytes is mediated by the catalytic p110 δ subunit,^{1,2} treatment with p110 δ -specific inhibitor could offer a new treatment with possibly higher efficiency and less unwanted side effects.

Overall, our study indicates that the splice donor and splice acceptor sites of exon 11 (coding exon 10) of the *PIK3R1* gene should be sequenced in patients with sporadic or autosomal dominant primary immunodeficiencies associated with lymphadenopathies, growth retardation, antibody deficiency (especially HIGM), B-cell lymphopenia with an increased percentage of transitional B cells, and naive CD4 and CD8 T-cell lymphopenia.

Finally, our study also indicates the need for further prospective, large-cohort studies of APDS2 to identify clinical or laboratory biomarkers that predict disease severity and to document the effect of different treatment options.

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Clinical implications: APDS2/PASLI-R1 should be screened in patients with sporadic or autosomal dominant primary immunodeficiencies associated with lymphadenopathies, growth retardation, high IgM levels (HIGM-like syndrome), and B-cell lymphopenia with an increased percentage of transitional B cells and decreased naïve T-cell counts.

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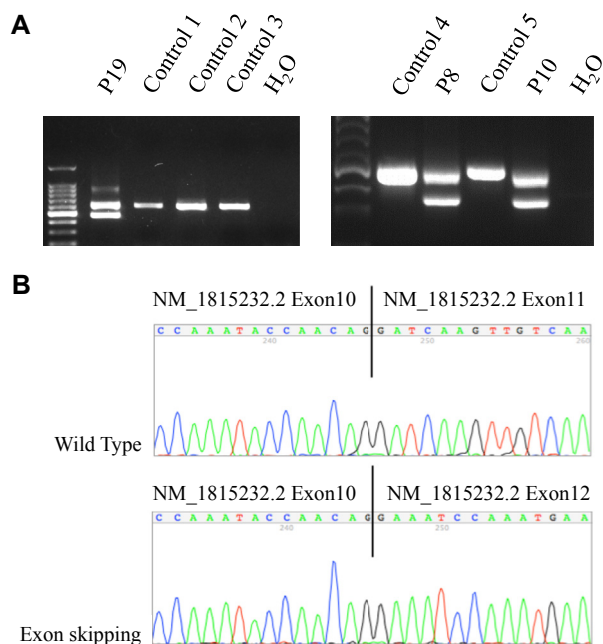


FIG E1. Novel splice acceptor and splice donor mutation at exon 11 (coding exon 10) of the *PIK3R1* gene can lead to exon skipping. **A**, RT-PCRs with primers flanking exon 11 of the *PIK3R1* gene with RNA extracted from patients (P19, PBLs; P8, fibroblasts; P10, T-cell blasts) and healthy subjects (control 1-3, PBLs; control 4, fibroblasts; control 5, T-cell blasts). P19 has a *de novo* mutation at the splice acceptor site (GRCh38; NM181523.2; C.1300 –1 position; G to C). P8 has a *de novo* mutation at the splice donor site (GRCh38; NM181523.2; C.1425 +2 position T to G). P10 has a 2-nt deletion at the splice donor site (GRCh38; NM181523.2; C.1425 +2,3 position; TG deletion). **B**, Sequencing chromatogram showing skipping of exon 11 (coding exon 10). Sequencing was performed with PCR products amplified from cDNA from P28. P28 has a *de novo* mutation at the splice donor site (GRCh38; NM181523.2; C.1425 +2 position T to A).

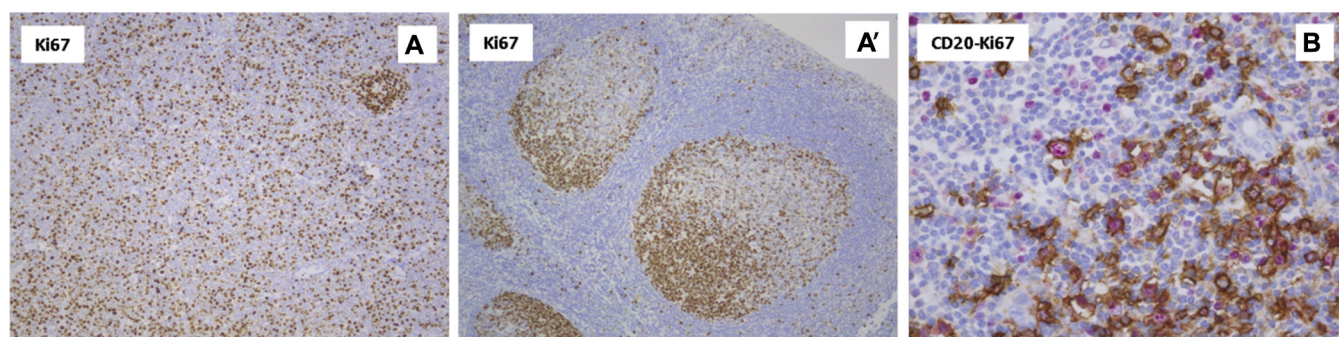


FIG E2. Large B cells in the interfollicular area are in cycle. Tonsil histology of patients with APDS2 showed numerous Ki67⁺ cells outside the positive germinal center (**A**; $\times 10$ magnification) in comparison with a control (**A'**; $\times 10$ magnification). Double staining (**B**; CD20: *brown*; Ki67: *red*; $\times 40$ magnification) showed that large B cells in the interfollicular area were in cycle.

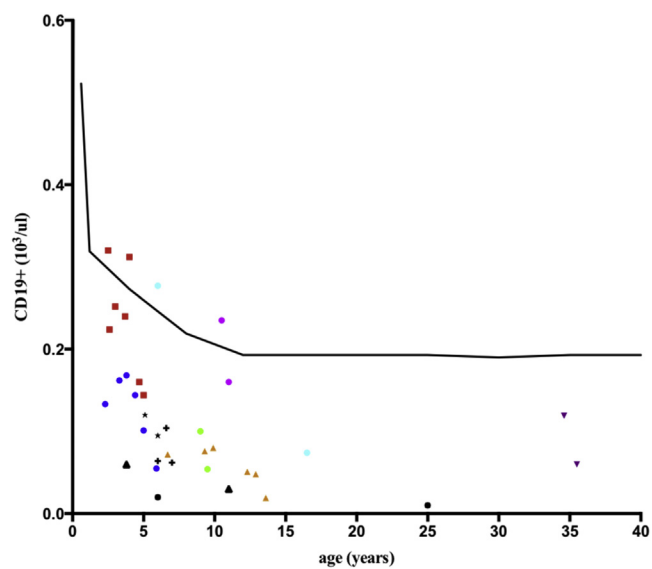


FIG E3. B-cell counts over time. Each *symbol* represents a patient with APDS2. *Solid line*, Lower reference value.

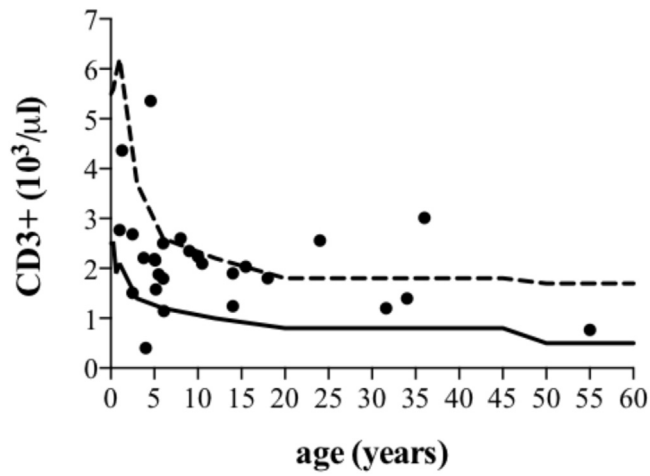


FIG E4. CD3 T-cell counts before onset of any therapies. *Solid line*, Lower reference value; *dashed line*, upper reference value.

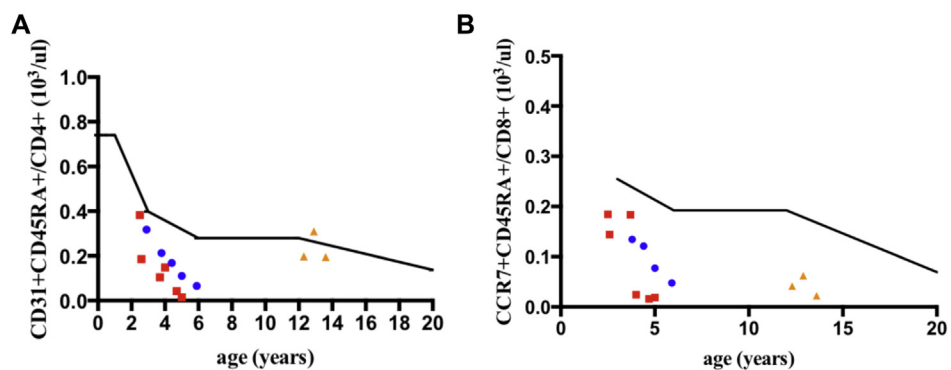


FIG E5. Development of naive T cells over time. Numbers of naive CD4 (**A**) and naive CD8 (**B**) T cells are shown. Each *symbol* represents a patient with APDS2. *Solid line*, Lower reference value.

TABLE E1. B-lymphocyte subsets and immunoglobulin serum levels at initial assessment

Patient ID	Age (y)	IgG (g/L)	IgA (g/L)	IgM (g/L)	CD19 ⁺ (/μL)	CD19 ⁺ /CD21 ⁺	CD24++ (%)	CD19 ⁺ /CD38 ⁺	IgM ⁺ (%)	Memory B cells (%)	Switched memory B cells (%)	MZB cells (%)
P1	1	2.2	0.23	3.3							11	
P2	2.5	0.07	0.06	3.67	162		26			8	25	45
P3a	34	22	0.05	2.26								
P3b	2.5	0	0	2.89	224					8	41	
P4a	NA											
P4b	5.5	3.64	<0.07	1.66	144							
P5	4	1.2	0	0	0							
P6	6	0.2	0.03	1.5	20							
P7	NA											
P8	5.1	1.4	0.07	2.7	120					11	5	6
P9	3.8	0.33	0.07	6.6	60					37	26	11
P10	1.3	0.2	0.1	1.44	192							
P11	NA											
P12	NA											
P13	12	5.64	0.09	2.38								
P14	6	4.6	0.1	1.05	277							
P15	24	1.75	0.254	8.87	48							
P16	9	2.8	0	>2.8	100							
P17	10.5	4.5	0.06	9.35	233							
P18	4.5	3.68	0.1	4.68								
P19	17	3.37	0	2.19						2.5	5.1	
P20a	NA											
P20b	8	3.5	0	0.15								
P21	7	0.16	0.25	1.15								
P22	NA											
P23a	NA											
P23b	NA											
P24	NA											
P25	4.9	0.24	0.01	0.14	0.52							
P26	4.6	5.79	1.81	5.42	149			15.24		2.3	1.3	0.2
P27a	31.6	5.09	0.06	2.62	250							
P27b	3	0.1	0.06	10								
P28	5.2	9.34	0.06	0.55	180							
P29	5	0.9	0.07	1.16	228			65.8		0.8	0.5	0.3
P30	1	2.38	0.04	3.06	107		70.1			9.2		3.9
P31	18	4.7	0.1	2.3	55					14	0.0	

NA, Not applicable.

TABLE E2. B-lymphocyte subsets and immunoglobulin serum levels at later assessment

Patient ID	Age (y)	IgG (g/L)	IgA (g/L)	IgM (g/L)	CD19 ⁺ (/μL)	CD19/CD21 ⁺ CD24 ⁺⁺ (%)	CD19 ⁺ /CD38 ⁺⁺ IgM ⁺⁺ (%)	Memory B cells (%)	Switched memory B cells (%)	MZB cells (%)	Treatment with rituximab
P1	12	16.97§	0.11	1.91	108	11	12	22	13	2	7.5 y after rituximab
P2	5.5	14.6§	0.05	1.73	105	39	39	8	3	2	
P3a	34.6	22§	0.05	2.26	119	11		64.5	42	34	
P3b	4	8.28§	0.05	4.81	120	33	31	9	6	2	
P4a	55	5.3	4.27	1.43	64		2	39	22	17	
P4b	18.5	0.3	0.04	10.63	58		5	50	45	13	
P5	26*	5§	0	0	0						
P6	25	9.27§	0.04	0.04	10						
P7	22				38			14			
P8	6	6.84§			95		23	14	7	4	
P9	11	9.06§	0.07	2.81	30			21	12	9	
P10	NA										
P11	25	0.03	0.05	0.42							
P12	NA										
P13	18	10.08§	0.05	5	81	50.8		25.5	18.6	6.9	
P14	16.5	5.68§	0.1	0.1	74						
P15	NA										
P16	9.5	5.97§	0	0.77	54	9.2	42	27	19	9.1	
P17	11	8.7§	0.06	10.5	160	19.2	45.7	8.5	3.1	4.2	
P18	13	11.26§	0.05	1.85	19	20		18	93		
P19	27†						9.23	2.5	50	33.3	
P20a	NA										1 y after rituximab
P20b	26†	5.8§	0	0.16	0						
P21	7.2	0.16	0.25	1.15	64	40	37	0.6	0.6	3	
P22	33.7‡	15.11§	0.05	16.46	122						
P23a	16	9.5§	0	0	0						
P23b	NA										
P24	14				13.8						
P25	6.1	4.45§	0.13	0.003							
P26	4.6	5.79	1.81	5.42	149		15.24	2.29	1.3	0.2	
P27a	44.4‡	10.3§	0.06	0.19	1						
P27b	15	7§	0.06	0.05	32			15	2	12	2 y after rituximab
P28	25*	16.3§	0.06	0.42	33	68		28	9.8	13.7	
P29	9	13§	0.06	0.63			40.5		1.9	4.3	
P30	2.2						9.23	2.5	50	33.33	
P31	NA										

NA, Not applicable.

*Less than 2 years after chemotherapy.

†More than 10 years after chemotherapy for lymphoma.

‡Receiving immunosuppressive drugs.

§Receiving immunoglobulin replacement.

TABLE E3. T-lymphocyte subsets at initial assessment

Patient ID	Age (y)	CD3 ⁺ (/μL)	CD4 ⁺ (/μL)	CD8 ⁺ (/μL)	CD16 ⁺ CD56 ⁺ (/μL)
P1	1				
P2	2.5	1512	432	576	126
P3a	34	1394	833	476	
P3b	2.5	2688	928	1600	224
P4a	NA				
P4b	5.5	1878	771	953	285
P5	4	400	100	300	100
P6	6	2500	1000	1400	350
P7	15.5	2035	496	1251	
P8	5.1	2160	730	1020	350
P9	3.8	2210	660	1320	120
P10	1.3	4363	2349	1630	288
P11	NA				
P12	NA				
P13	12				
P14	6	1793	861	780	633
P15	24	2560	482	1850	134
P16	9	2350	870	1330	190
P17	10.5	2100	470	1363	2350
P18	4.5	1900	779	741	
P19	17				
P20a	36				
P20b	NA				
P21	NA				
P22	6				
P23a	16	2661	2233	942	430
P23b	NA				
P24	NA				
P25	4.9	1144	195	789	115
P26	4.6	5357	1243	3868	764
P27a	31.6	1200	880	550	210
P27b	NA				
P28	5.2	1580	730	950	200
P29	5	2187	696	1439	285
P30	1	2769	781	1687	835
P31	18	1800	400	1380	320

NA, Not applicable.

TABLE E4. T-lymphocyte subsets at later assessment

Age (y)	CD3 ⁺ (/μL)	CD4 ⁺ (/μL)	Naive CD4 CD31 ⁺ CD45RA/CD4 ⁺ (%)	Naive CD4 CD31 ⁺ CD45RA/CD4 ⁺ (/μL)	CD8 ⁺ (/μL)	Naive CD8 CCR7 ⁺ CD45RA ⁺ /CD8 ⁺ (%)	Naive CD8 CCR7 ⁺ CD45RA ⁺ /CD8 ⁺ (/μL)	CD16 ⁺ CD56 ⁺ (/μL)	Senescent T cell (%)
12	1,440	540	7	38	702	1	7		19.5
5.5	957	330	20	66	341	14	48	55	16
34.6	1,394	833	4	33	476	4	19	187	16.3
4	3,432	780			2,457		25	156	8.89
55	764	420	24	103	337			356	
18.5	2,107	617	7	42	1,330	5	63	138	
26*	280	20	0	0	260			20	
25	1,940	770			1,140			240	
22	1,714	452	5	23	1,036	4	41	132	23.4
6	11,558	513	17	87	760	2	15	228	21.3
11	1,440	530	22	140	750			30	
NA									
25		650			1,400				
NA									
18	704	200	8.5	17	415			1,031	
16.5	2,067	886			1,187			14	
NA									
9.5	2,155	488	3.2	12	1,384			56	
11	2,930	530	4.7	25	2,390			1,650	
13	2,040	1,032	30	310	888	7	62	312	26.4
NA									
36†	3,015	385	4	16	2,554	1	23	157	
26‡	2,600	400	0.5	2	2,200	0	0	100	
7.2	1,312	432	31	135	512	7	37		
33.7§	4,488	1,079			3,210				
16	2,233	942			1,318			430	
NA									
14	1,242	869			276				
NA									
4.6	5,357	1,243	29	363	3,868			764	
44.4§	455	228			218			6	
15	965	297	35	103	619			283	
25*	3,302	404			2,898			370	
9	3,839	2,614	13	347	1,225			124	
NA									
NA									

*Less than 2 years after chemotherapy.

†More than 10 years after chemotherapy and radiotherapy for lymphoma.

‡More than 10 years after chemotherapy for lymphoma.

§Receiving immunosuppressive drugs.